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International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : H01J 49/04

A1

(11) International Publication Number: WO 98/59362

(43) International Publication Date: 30 December 1998 (30.12.98)

(21) International Application Number: PCT/US98/12908

(22) International Filing Date: 19 June 1998 (19.06.98)

(30) Priority Data:
60/054,333 20 June 1997 (20.06.97) US
60/067,484 1 December 1997 (01.12.97) US

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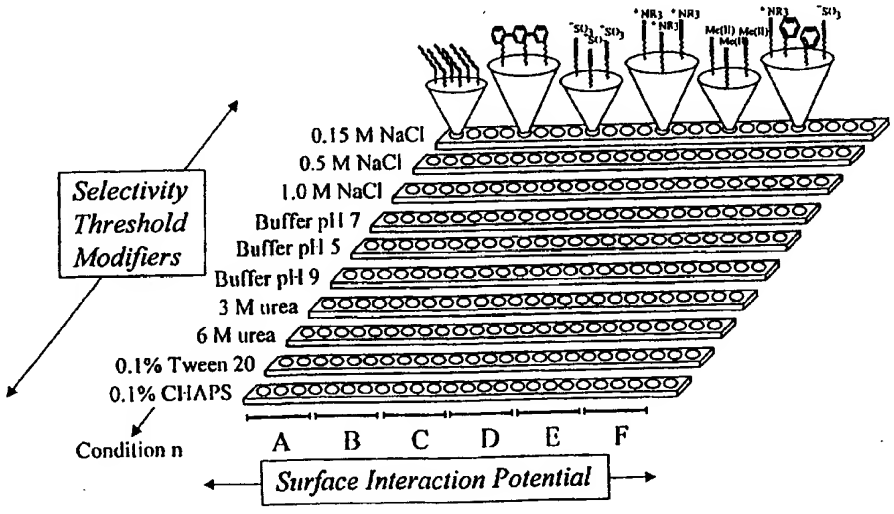
(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published

With international search report.

Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: RETENTATE CHROMATOGRAPHY AND PROTEIN CHIP ARRAYS WITH APPLICATIONS IN BIOLOGY AND MEDICINE



(57) Abstract

This invention provides methods of retentate chromatography for resolving analytes in a sample. The methods involve adsorbing the analytes to a substrate under a plurality of different selectivity conditions, and detecting the analytes retained on the substrate by desorption spectrometry. The methods are useful in biology and medecine, including clinical diagnostics and drug discovery.

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RETENTATE CHROMATOGRAPHY AND PROTEIN CHIP ARRAYS WITH APPLICATIONS IN BIOLOGY AND MEDICINE

CROSS-REFERENCE TO RELATED APPLICATION

This application claims the benefit of the priority dates of co-pending application 60/054,333 filed June 20, 1997 and co-pending application 60/067,484 filed December 1, 1997, the contents of which are incorporated herein by reference in their entirety.

FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

Not applicable.

BACKGROUND OF THE INVENTION

This invention relates to the field of separation science and analytical biochemistry.

The methods of this invention have applications in biology and medicine, including analysis of gene function, differential gene expression, protein discovery, cellular and clinical diagnostics and drug screening.

Cell function, both normal and pathologic, depends, in part, on the genes expressed by the cell (i.e., gene function). Gene expression has both qualitative and quantitative aspects. That is, cells may differ both in terms of the particular genes expressed and in terms of relative level of expression of the same gene. Differential gene expression can be manifested, for example, by differences in the expression of proteins encoded by the gene, or in post-translational modifications of expressed proteins. For example, proteins can be decorated with carbohydrates or phosphate groups, or they can be processed through peptide cleavage. Thus, at the biochemical level, a cell represents a complex mixture of organic biomolecules.

One goal of functional genomics ("proteomics") is the identification and characterization of organic biomolecules that are differentially expressed between cell

types. By comparing expression one can identify molecules that may be responsible for a particular pathologic activity of a cell. For example, identifying a protein that is expressed in cancer cells but not in normal cells is useful for diagnosis and, ultimately, for drug discovery and treatment of the pathology. Upon completion of the Human Genome Project, all the human genes will have been cloned, sequenced and organized in databases. In this "post-genome" world, the ability to identify differentially expressed proteins will lead, in turn, to the identification of the genes that encode them. Thus, the power of genetics can be brought to bear on problems of cell function.

Differential chemical analyses of gene expression and function require tools that can resolve the complex mixture of molecules in a cell, quantify them and identify them, even when present in trace amounts. However, the current tools of analytical chemistry for this purpose are limited in each of these areas. One popular biomolecular separation method is gel electrophoresis. Frequently, a first separation of proteins by isoelectric focusing in a gel is coupled with a second separation by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The result is a map that resolves proteins according to the dimensions of isoelectric point (net charge) and size (i.e., mass). However useful, this method is limited in several ways. First, the method provides information only about two characteristics of a biomolecule -- mass and isoelectric point ("pI"). Second, the resolution power in each of the dimensions is limited by the resolving power of the gel. For example, molecules whose mass differ by less than about 5% or less than about 0.5 pI are often difficult to resolve. Third, gels have limited loading capacity, and thus sensitivity; one may not be able to detect biomolecules that are expressed in small quantities. Fourth, small proteins and peptides with a molecular mass below about 10-20 kDa are not observed.

Other analytical methods may overcome one or more of these limitations, but they are difficult to combine efficiently. For example, analytical chromatography can separate biomolecules based on a variety of analyte/adsorbent interactions, but multi-dimensional analysis is difficult and time consuming. Furthermore, the methods are limited in sensitivity.

Clinical diagnostics requires the ability to specifically detect known markers of disease. However, the development of such diagnostics is hampered by the time necessary to prepare reagents that specifically bind to markers, or that can discriminate the marker in a complex mixture.

Drug discovery requires the ability to rapidly screen agents that modulate ligand/receptor interactions. Often the rate-limiting step in such screens is the ability to detect the ligand/receptor interaction. Thus, rapid and specific methods for identifying binding events would be an advance in the art.

Until now, the process from identifying a potential marker or member of a ligand/receptor pair to producing an agent that specifically binds the marker or member has been difficult. In one method, normal and diseased tissue are compared to identify mRNA species or expressed sequence tags ("ESTs") that are elevated or decreased in the diseased tissue. These species are isolated and the polypeptides they encode are produced through routine methods of recombinant DNA. Then, the polypeptides are isolated and used as immunogens to raise antibodies specific for the marker. The antibodies can be used in, for example, ELISA assays to determine the amount of the marker in a patient sample.

This process is long and tedious. It can take nine months to a year to produce such antibodies, with much of the time being spent on developing protocols to isolate a sufficient quantity of the polypeptide for immunization. Furthermore, the method relies on the hope that differences in RNA expression are expressed as differences in protein expression. However, this assumption is not always reliable. Therefore, methods in which differentially expressed proteins are detected directly and in which specific ligands could be generated in significantly shorter time would be of great benefit to the field.

Thus, tools for resolving complex mixtures of organic biomolecules, identifying individual biomolecules in the mixture and identifying specific molecular recognition events involving one or more target analytes are desirable for analytical biochemistry, biology and medicine.

SUMMARY OF THE INVENTION

This invention provides devices and methods for retentate chromatography. Retentate chromatography is a combinatorial method to provide high information resolution of analytes in complex mixtures through the use of multi-dimensional separation methods. It provides a unified analyte detection and functional analysis capability for biology and medicine that is characterized by a single, integrated operating system for the direct detection of analyte expression patterns associated with gene

function, protein function, cell function, and the function of whole organisms. In one aspect, this invention provides a unified operating system for the discovery or diagnosis of gene function, protein function, or the function of entire macromolecular assemblies, cells, and whole organisms.

More particularly, analytes can be resolved in a variety of two-dimensional formats, thereby providing multi-dimensional information. Analytes are first separated in at least two different first dimensions based on their ability to be adsorbed to a stationary phase under at least two different selectivity conditions, such as anionic/cationic potential, hydrophobicity/hydrophilicity, or specific biomolecular recognition. Then the analytes are separated in a second dimension based on mass by desorption spectrometry (e.g., laser desorption mass spectrometry), which further provides detection of the separated analytes. The nature of the adsorbent to which the analytes adsorb provides physico-chemical information about the analyte.

Thus, this invention provides a molecular discovery and diagnostic device that is characterized by the inclusion of *both* parallel and multiplex analyte processing capabilities. Because analytes are directly detected, the invention enables the simultaneous transmission of two or more independent target analyte signals from the same "circuit" (i.e., addressable "chip" location) during a single unit operation.

Retentate chromatography is distinct from conventional chromatography in several ways. First, in retentate chromatography, analytes which are retained on the adsorbent are detected. In conventional chromatographic methods analytes are eluted off of the adsorbent prior to detection. There is no routine or convenient means for detecting analyte which is not eluted off the adsorbent in conventional chromatography. Thus, retentate chromatography provides direct information about chemical or structural characteristics of the retained analytes. Second, the coupling of adsorption chromatography with detection by desorption spectrometry provides extraordinary sensitivity, in the femtomolar range, and unusually fine resolution. Third, in part because it allows direct detection of analytes, retentate chromatography provides the ability to rapidly analyze retentates with a variety of different selectivity conditions, thus providing rapid, multi-dimensional characterization of analytes in a sample. Fourth, adsorbents can be attached to a substrate in an array of pre-determined, addressable locations. This allows parallel processing of analytes exposed to different adsorbent sites (i.e., "affinity sites" or "spots") on the array under different elution conditions.

Retentate chromatography has many uses in biology and medicine. These uses include combinatorial biochemical separation and purification of analytes, the study of differential gene expression and molecular recognition events, diagnostics and drug discovery.

One basic use of retentate chromatography as an analytical tool involves exposing a sample to a combinatorial assortment of different adsorbent/eluant combinations and detecting the behavior of the analyte under the different conditions. This both purifies the analyte and identifies conditions useful for detecting the analyte in a sample. Substrates having adsorbents identified in this way can be used as specific detectors of the analyte or analytes. In a progressive extraction method, a sample is exposed to a first adsorbent/eluant combination and the wash, depleted of analytes that are adsorbed by the first adsorbent, is exposed to a second adsorbent to deplete it of other analytes. Selectivity conditions identified to retain analytes also can be used in preparative purification procedures in which an impure sample containing an analyte is exposed, sequentially, to adsorbents that retain it, impurities are removed, and the retained analyte is collected from the adsorbent for a subsequent round.

One aspect of the invention is that each class or type of molecular recognition event (e.g., target adsorbent-target analyte interaction), characterized by a particular selectivity condition at an addressable location within the array, is detected directly while the associated molecules are still localized (i.e., "retained") at the addressable location. That is, selection and detection, by direct means, does not require elution, recovery, amplification, or labeling of the target analyte.

Another aspect of the present invention is that the detection of one or more desirable molecular recognition events, at one or more locations within the addressable array, does not require removal or consumption of more than a small fraction of the total adsorbent-analyte. Thus, the unused portion can be interrogated further after one or more "secondary processing" events conducted directly *in situ* (i.e., within the boundary of the addressable location) for the purpose of structure and function elucidation, including further assembly or disassembly, modification, or amplification (directly or indirectly).

Adsorbents with improved specificity for an analyte can be developed by an iterative process, referred to as "progressive resolution," in which adsorbents or eluants proven to retain an analyte are tested with additional variables to identity

combinations with better binding characteristics. Another method allows the rapid creation of substrates with antibody adsorbents specific for an analyte. The method involves docking the analyte to an adsorbent, and screening phage display libraries for phage that bind the analyte.

Retentate chromatography has uses in molecular and cellular biology, as well. Analytes that are differentially present in two samples (e.g., differentially expressed proteins in two cell extracts) can be identified by exposing the samples to a variety of adsorbent/eluant combinations for analysis by desorption spectrometry, thereby making use of the high information resolving power of the system that other separation and detections systems cannot match. Unknown target proteins can be identified by determining physicochemical characteristics, including molecular mass, based on the chemical characteristics of the adsorbent/eluant combination, and this information can be used to screen databases for proteins having similar profiles.

The methods in separation biochemistry and the adsorbents produced from these methods, are useful in diagnostics. More particularly, adsorbents, either chemical or biospecific, can be developed to detect important diagnostic markers. In certain embodiments, a substrate can have an array of adsorbent spots selected for a combination of markers diagnostic for a disease or syndrome.

Retentate chromatography also is useful in drug discovery. One member of a receptor/ligand pair is docked to an adsorbent, and its ability to bind the binding partner is tested in the presence of the agent. Because of the rapidity with which adsorption can be tested, combinatorial libraries of agents can be easily tested for their ability to modulate the interaction.

In one aspect this invention provides a method for high information resolution of at least one analyte in a sample. The method is a combinatorial separation method that includes separation and detection of multiple analytes in parallel. The method comprises the steps of a) exposing the analyte to at least two different selectivity conditions, each selectivity condition defined by the combination of an adsorbent and an eluant, to allow retention of the analyte by the adsorbent; and b) detecting retained analyte under the different selectivity conditions by desorption spectrometry. Detection of retained analyte under the different selectivity conditions provides a high information resolution of the analyte.

In one embodiment each different selectivity condition is defined at a different predetermined, addressable location for parallel processing. In another embodiment, the method comprises the steps of i) exposing the analyte to a first selectivity condition at a defined location to allow retention of the analyte by the adsorbent; ii) detecting retained analyte under the first selectivity condition by desorption spectrometry; iii) washing the adsorbent under a second, different selectivity condition at the defined location to allow retention of the analyte to the adsorbent; and iv) detecting retained analyte under the second selectivity condition by desorption spectrometry.

In another embodiment the analyte is an organic biomolecule, a multimeric molecular complex or macromolecular assembly. In another embodiment the organic biomolecule is an enzyme, an immunoglobulin, a cell surface receptor or an intracellular receptor.

In another embodiment the adsorbent comprises an anion, a cation, a hydrophobic interaction adsorbent, a polypeptide, a nucleic acid, a carbohydrate, a lectin, a dye, a reducing agent, a hydrocarbon or a combination thereof. In another embodiment the adsorbent is attached to a substrate comprising glass, ceramic, a magnetic material, an organic polymer, a conducting polymer, a native biopolymer, a metal or a metal coated with an organic polymer. In another embodiment the adsorbent is in the form of a microemulsion, a latex, a layer or a bead. In another embodiment the locations on the substrate are arranged in a line or an orthogonal array. In another embodiment the adsorbents are located on a substrate at different locations before the analytes are exposed to the selectivity conditions. In another embodiment the adsorbents are located on a substrate at different locations after the analytes are exposed to the selectivity conditions. In another embodiment the different selectivity conditions comprise different binding conditions or different elution conditions.

In another embodiment the step of detecting comprises detecting the mass of the analyte by laser desorption mass spectrometry.

In another embodiment the selectivity conditions are selected to optimize retention of analyte by an adsorbent. In another embodiment the at least one analyte is more than one analyte. In another embodiment the plurality of selectivity conditions are defined by at different adsorbents and the same eluant.

Another embodiment further comprises the step of providing a substrate comprising adsorbents at addressable locations, each adsorbent being an adsorbent from a

selectivity condition identified to retain the analyte. In another embodiment the elution conditions differ according to pH, buffering capacity, ionic strength, a water structure characteristic, detergent type, detergent strength, hydrophobicity or dielectric constant. In another embodiment the plurality of selectivity conditions are defined by the same eluant.

In another embodiment this invention provides a method for sequential extraction of analytes from a sample. This is a combinatorial, serial separation and purification development method for multiple analytes in parallel. The method comprises the steps of a) exposing a sample comprising analytes to a first selectivity condition to allow retention of analytes by a first adsorbent and to create un-retained sample; b) collecting the un-retained sample comprising analytes, exposing the un-retained sample to a second selectivity condition to allow retention of analytes by a second adsorbent and to create un-retained sample; and c) detecting retained analyte under the different selectivity conditions by desorption spectrometry.

In another aspect this invention provides a substrate for desorption spectrometry comprising an adsorbent whose binding characteristics vary in a gradient along one or more linear axes.

In another aspect this invention provides a method for progressively identifying a selectivity condition with improved resolution for an analyte in a sample. The method comprises the steps of: (a) identify a selectivity condition that retains an analyte in a sample by (i) exposing a sample to a set of selectivity conditions, each selectivity condition defined by at least one binding characteristic and at least one elution characteristic; (ii) detecting analyte retained under each selectivity condition by desorption spectrometry; and (iii) identifying a selectivity condition that retains the analyte; and (b) identifying a selectivity condition with improved resolution for the analyte by: (i) selecting at least one binding characteristic or elution characteristic from the identified selectivity condition and adding it to a selectivity characteristic constant set; (ii) exposing the sample to a modified set of selectivity conditions wherein each selectivity condition in the modified set comprises (1) the selectivity characteristics in the constant set and (2) a binding characteristic or elution characteristic that is not in the constant set; and (iii) identifying a selectivity condition from the modified set by desorption spectrometry that retains the analyte with improved resolution compared with a prior identified selectivity condition. One embodiment comprises the step of repeating

step (b) at least once. Another embodiment comprises repeating steps (b) until a selectivity condition is identified that retains only the target analyte from the sample.

In another aspect this invention provides a substrate for desorption spectrometry comprising an adsorbent from a selectivity conditions identified to resolve an analyte by the method of progressive resolution. In one embodiment the substrate comes in the form of a kit further comprising an eluant from the selectivity condition or instructions on using the eluant in combination with the adsorbent.

In another aspect this invention provides a method for preparative purification an analyte from an impure sample. The method comprises the steps of a) exposing the sample to a substrate under a plurality of different selectivity conditions; detecting retained analyte under the different selectivity conditions by desorption spectrometry; and identifying selectivity conditions under which the analyte is retained; b) purifying the analyte by repeating, for a plurality of different identified selectivity conditions, a sequence of steps comprising i) exposing the sample to an adsorbent under the identified selectivity condition to allow retention of the analyte by the adsorbent; ii) separating the analyte from an impurity that is not retained by the substrate; and iii) collecting the analyte from the adsorbent.

In another aspect this invention provides a method for preparing a substrate for detecting at least one analyte in a sample. This method is a combinatorial method for the design and identification of analyte-specific adsorbents. It is useful in detecting target analytes. The method comprises the steps of a) exposing the sample to at least two different selectivity conditions, each selectivity condition defined by the combination of an adsorbent and an eluant, to allow retention of the analyte by the adsorbent; b) identifying by desorption spectrometry at least one selectivity condition under which the analyte is retained; and c) preparing a substrate comprising at least one adsorbent of an identified selectivity condition. In one embodiment, the step of identifying comprises identifying at least one selectivity condition under which a plurality of analytes are retained. In another embodiment the step of preparing comprises preparing a substrate comprising a plurality of adsorbents that retain the analyte under an elution condition as a multiplex adsorbent.

In another aspect this invention provides a method of diagnosing in a subject a disease characterized by at least one diagnostic marker. This is a combinatorial method for simultaneous detection of multiple diagnostic markers. The method

comprises the steps of a) providing a substrate for use in desorption spectrometry that comprises at least one addressable location, each addressable location comprising an adsorbent that resolves at least one of the diagnostic markers under an elution condition; b) exposing the substrate to a biological sample from the subject under the elution condition to allow retention of the diagnostic marker; and c) detecting retained diagnostic marker by desorption spectrometry. Detecting retained diagnostic marker provides a diagnosis of the disease.

In another aspect this invention provides a kit for detecting an analyte in a sample comprising (1) a substrate for use in desorption spectrometry that comprises at least one addressable location, each addressable location comprising an adsorbent that resolves an analyte under a selectivity condition comprising the adsorbent and an eluant, and (2) the eluant or instructions for exposing the sample to the selectivity condition. In one embodiment the kit is characterized by a plurality of diagnostic markers and the substrate comprises a plurality of addressable locations, each addressable location comprising an adsorbent that resolves at least one of the diagnostic markers.

In another aspect this invention provides a substrate for desorption spectrometry comprising at least one adsorbent in at least one addressable location wherein the at least one adsorbent resolves a plurality of diagnostic markers for a pathological condition from a patient sample.

In another aspect this invention provides a method for selecting identity candidates for an analyte protein. This method is a combinatorial method for protein identification based on at least two physico-chemical properties. The method comprises the steps of a) determining a value set specifying match parameters for at least a first and second physico-chemical characteristic of a protein analyte in a sample by i) exposing the analyte to a plurality of different selectivity conditions, wherein adsorption of the protein analyte to the substrate is mediated by a basis of attraction that identifies a physico-chemical characteristic of the protein analyte; and ii) detecting retained analyte under the different selectivity conditions by desorption spectrometry; and b) performing, in a programmable digital computer, the steps of i) accessing a database comprising, for each member of a set of reference polypeptides, a value set specifying at least a first and second physico-chemical characteristic of the reference polypeptides; ii) inputting the value set specifying the physico-chemical characteristics of the protein analyte; iii) sorting from the database, reference polypeptides having value sets within the match

parameters. The sorted reference polypeptides provide identity candidates for the protein analyte. Unsorted references polypeptides are those excluded as identity candidates.

In another aspect this invention provides a method for sequentially retaining analytes. This method is a multimeric macromolecular or supramolecular assembly monitoring method. It is useful as a method for drug discovery by molecular recognition interference. The method comprises the steps of a) exposing a first sample to a primary adsorbent and to an eluant to allow retention of a first analyte by the adsorbent, and detecting the adsorbed analyte by desorption spectrometry, whereby the retained first analyte becomes a secondary adsorbent; b) exposing a second sample to the secondary adsorbent and to an eluant to allow retention of a second analyte by the secondary adsorbent, and detecting the adsorbed second analyte by desorption spectrometry, whereby the retained second analyte becomes a tertiary adsorbent.

In another aspect this invention provides a method of detecting an enzyme in a sample. The method comprises the steps of: a) providing a solid phase comprising an adsorbent and an enzyme substrate bound to the adsorbent, wherein the activity of the enzyme on the enzyme substrate produces a product having a characteristic molecular mass; b) exposing the substrate to the sample; and c) detecting the product by desorption spectrometry. Detecting the product provides a detection of the enzyme.

In another aspect this invention provides a method for determining whether an analyte is differentially present (e.g., differentially expressed) in a first and second biological sample. The method is useful for combinatorial method for differential gene expression monitoring by differential protein display. The method comprises the steps of a) determining a first retention map for the analyte in the first sample for at least one selectivity condition; b) determining a second retention map for the analyte in the second sample for the same selectivity condition; and c) detecting a difference between the first and the second retention maps. A difference in the retention maps provides a determination that the analyte is differentially present in first and second samples.

In one embodiment the method is for determining whether a protein is differentially expressed between two different cells, and the first and second samples comprise the cells or material from the cells. In another embodiment the method if for determining whether an agent alters the expression of a protein in a biological sample further comprising the step of administering the agent to a first biological sample but not to a second biological sample. In another embodiment the first biological sample derives

from a healthy subject and the second biological sample is from a subject suffering from a pathological condition. The sample can be selected from, for example, blood, urine, serum and tissue. Analytes that are found to be increased in samples from pathological subjects are candidate diagnostic markers. Generally, confirmation of a dianostic marker involves detection of the marker in many subjects.

In another aspect this invention provides a method for identifying a ligand for a receptor. The method comprises the steps of: a) providing a substrate comprising an adsorbent wherein the receptor is bound to the adsorbent; b) exposing the bound receptor to a sample containing the ligand under conditions to allow binding between the receptor and the ligand; and c) detecting bound ligand by desorption spectrometry.

In another aspect this invention provides a screening method for determining whether an agent modulates binding between a target analyte and an adsorbent. This is a combinatorial method for drug discovery. The method comprises the steps of a) providing a substrate comprising an adsorbent to which the target analyte binds under an elution condition; b) exposing the substrate to the target analyte and to the agent under the elution condition to allow binding between the target analyte and the adsorbent; c) detecting an amount of binding between the target analyte and the adsorbent by desorption spectrometry; and d) determining whether the measured amount is different than a control amount of binding when the substrate is exposed to the target analyte under the elution condition without the agent. A difference between the measured amount and the control amount indicates that the agent modulates binding.

In one aspect, this invention provides a method of detecting a genetic package containing a polynucleotide that encodes a polypeptide agent that specifically binds to a target adsorbent. This is, in one aspect, a combinatorial method for selecting analyte-specific phage from a display library, including the use of target proteins isolated by retentate mapping or target proteins generated *in situ* by *in vitro* transcription and translation. The method comprises the steps of: a) providing a substrate comprising a target adsorbent; b) providing a display library that comprises a plurality of different genetic packages, each different genetic package comprising a polynucleotide that comprises a nucleotide sequence that encodes a polypeptide agent, and each different genetic package having a surface on which the encoded polypeptide agent is displayed; c) exposing the substrate to the display library under elution conditions to allow specific binding between a polypeptide agent and the target adsorbent; whereby a genetic package

comprising the polypeptide agent is retained on the substrate; and d) detecting a genetic package retained on the substrate by desorption spectrometry.

In one embodiment of this method, the display library is a phage display library. In another embodiment the phage is M13. In another embodiment the polypeptide is a single chain antibody. In another embodiment the target analyte is a polypeptide analyte that is differentially expressed between cells of different phenotypes. In another embodiment the substrate comprises a cell or cell membrane.

In one embodiment, the step of providing the substrate comprising the target adsorbent comprises the steps of: i) providing a substrate comprising an adsorbent, wherein the adsorbent retains a target analyte under an elution condition; and ii) exposing the adsorbent to the target analyte under the elution condition to allow retention of the target analyte by the adsorbent, whereby the target analyte becomes the target adsorbent. In one embodiment, the target analyte is a target polypeptide and the step of ii) exposing the adsorbent comprises the step of producing the target polypeptide *in situ* on the adsorbent by *in vitro* translation of a polynucleotide encoding the target polypeptide, and can further comprise amplifying the polynucleotide sequence *in situ* on the substrate.

In another embodiment the substrate comprises (1) an adsorbent that binds an anchoring polypeptide and (2) at least one target genetic package having a surface displaying the anchoring polypeptide and a target adsorbent polypeptide, the target genetic package comprising a polynucleotide that comprises a nucleotide sequence that encodes the target adsorbent, wherein the target genetic package is bound to the adsorbent through the anchoring polypeptide.

In another embodiment the method further comprises any of the following steps: sequencing the nucleotide sequence that encodes the polypeptide agent; isolating the retained genetic package or producing the polypeptide agent.

In another aspect this invention provides a substrate for desorption spectrometry comprising an adsorbent that binds an anchoring polypeptide displayed on a surface of a genetic package, wherein the surface of the genetic package further displays a target polypeptide and wherein the genetic package comprises a polynucleotide comprising a nucleotide sequence that encodes the target polypeptide.

In another aspect this invention provides a method for detecting translation of a polynucleotide. The method comprises the steps of: a) providing a substrate comprising an adsorbent for use in desorption spectrometry; b) contacting the substrate

with the polynucleotide encoding a polypeptide and with agents for *in vitro* translation of the polynucleotide, whereby the polypeptide is produced; c) exposing the substrate to an eluant to allow retention of the polypeptide by the adsorbent; and d) detecting retained polypeptide by desorption spectrometry. Detection of the polypeptide provides detection of translation of the polynucleotide.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 depicts a substrate containing a plurality of adsorbent spots in the form of a strip. The strip contains six different sets adsorbents classified according to a basis of attraction (hydrophobic, ionic, coordinate covalent and mixed function). The strip contains several spots for each type of adsorbent, allowing interrogation of the spots at different times with different eluants, or for archiving and subsequent analysis.

Fig. 2 depicts an orthogonal array of adsorbents (surface interaction potentials) in predetermined, addressable locations. The array also can take the form of a plate. The array includes various adsorbents. Upon exposure to the analyte, each strip can be washed by a variety of eluants (selectivity threshold modifiers). Analysis of retention under different selectivity conditions results in retention map or recognition profile.

Fig. 3 is a representation of the quantitative analysis of analytes by desorption of analyte from given locations on the array and quantitative detection of the desorbed analyte by laser desorption mass spectrometry.

Fig. 4A illustrates an example of a computer system used to execute software that can be used to analyze data generated by the present invention. Fig. 4A shows a computer system 1 which includes a monitor 3, screen 5, cabinet 7, keyboard 9, and mouse 11. Mouse 11 may have one or more buttons such as mouse buttons 13. Cabinet 7 houses a CD-ROM drive 15 and a hard drive (not shown) that may be utilized to store and retrieve computer programs including code incorporating the present invention. Although a CD-ROM 17 is shown as the computer readable storage medium, other computer readable storage media including floppy disks, DRAM, hard drives, flash memory, tape, and the like may be utilized. Cabinet 7 also houses familiar computer components (not shown) such as a processor, memory, and the like.

Fig. 4B shows a system block diagram of computer system 1 used to execute software that can be used to analyze data generated by the present invention. As

in Fig. 4A, computer system 1 includes monitor 3 and keyboard 9. Computer system 1 further includes subsystems such as a central processor 102, system memory 104, I/O controller 106, display adapter 108, removable disk 112, fixed disk 116, network interface 118, and speaker 120. Removable disk 112 is representative of removable computer readable media like floppies, tape, CD-ROM, removable hard drive, flash memory, and the like. Fixed disk 116 is representative of an internal hard drive, DRAM, or the like. Other computer systems suitable for use with the present invention may include additional or fewer subsystems. For example, another computer system could include more than one processor 102 (i.e., a multi-processor system) or memory cache.

Figs. 5A-5F show retention maps for lysozyme under selectivity conditions including six different adsorbents and several different eluants.

Figs. 6A-6B show the resolution at low and high molecular mass of analytes in human serum by an immobilized metal adsorbent.

Figs. 7A-7B show the resolution at low and high molecular mass of analytes in human serum by a variety of adsorbents using the same eluant.

Figs. 8A-8B show the resolution at low and high molecular mass of analytes in preterm infant urine by a variety of adsorbents using water as the eluant.

Fig. 9 shows resolution of analytes in preterm infant urine using a hydrophobic phenyl adsorbent and three different eluants, resulting in the discovery of selective retention of one of the analytes (*) by the Tween wash condition.

Fig. 10A-10D show the resolution of analytes in cell culture medium of two different breast cancer cell lines.

Fig. 11 shows a composite retention map of preterm infant urine exposed to selectivity conditions defined by six different adsorbents and three different eluants.

Fig. 12 shows a two-dimensional polyacrylamide gel (pI and apparent molecular mass) of preterm infant urine.

Fig. 13 shows a method of panning with phage display libraries for a phage having a surface protein that specifically binds to a target analyte. The substrate depicted at the top shows that even a few specifically bound phage can be detected by desorption spectrometry through the detection of the many coat proteins that phage contains. At the bottom, a substrate with several adsorbent spots is developed so that the target analyte is specifically bound. Phage are exposed to the spots. Bound phage are

detected by desorption spectrometry. Phage bound to another spot can be isolated and grown.

Fig. 14 shows how a ligand agent, in this case a single chain antibody, identified by a panning method can be used as an adsorbent to dock a target protein for use in protein-protein interaction studies. A target is purified *in situ* (spot 2) and used to pan a phage display library (spot 4). A single chain antibody is isolated and attached to a substrate (spot 6) as an adsorbent. The target is then adsorbed to the single chain antibody. The target is now docked for the study of protein-protein interactions (spot 8).

Fig. 15 shows a method for screening drug candidates for the ability to interfere with protein binding to a ligand, in this case a single-chain antibody. A single chain antibody specific for a target protein is docked to a spot on a substrate through, for example, an anti-phage antibody which, itself, can be docked through protein A or protein G. The single chain antibody is exposed to the target protein and to drug candidates. The ability of the drug to bind to the analyte protein and to interfere with ligand binding to analyte is monitored by desorption spectrometry.

Fig. 16 shows a method for screening drug candidates for the ability to interfere with protein binding to a ligand. The method is similar to that depicted in the previous figure, except one monitors the ability of the drug to interfere with analyte binding by binding, itself, to the ligand by desorption spectrometry.

Fig. 17 shows a method for screening drug candidates for the ability to interfere with target protein (Target protein 1) binding to a secondary ligand (Target Protein II). As in the previous two figures, the target is docked to the substrate becoming, itself, an adsorbent for the ligand. In this case, the analyte is docked through a single chain antibody. The target is then exposed to the ligand and to the drug candidates. The ability of the drug to interfere with binding between the analyte and the ligand (by, e.g., binding to the target analyte) is monitored by desorption spectrometry.

Fig. 18 depicts a flow chart beginning with the identification of differentially expressed mRNA or polypeptides and ending with the creation of a diagnostic platform for specifically binding the polypeptide for detection by desorption spectrometry.

Figs. 19A-19D show a retention map of *Hemophilus* lysate on an adsorbent array. Fig. 19A: anionic adsorbent; Fig. 19B: Normal phase adsorbent; Fig. 19C: Ni(II) adsorbent; Fig. 19D: Hydrophobic adsorbent.

Figs. 20A-20C show progressive resolution of an analyte in *Hemophilus* lysate. The adsorbent in each case was an anionic adsorbent. Fig. 20A: In a first step, after exposure to the sample, the spot was washed with 150 μl of 20 mM sodium phosphate, 0.5 M sodium chloride, pH 7.0. In a second step, the adsorbent and sodium phosphate characteristic of the eluant were added to a constant set of characteristics. A new elution characteristic was added. Fig. 20B: In addition to 20 mM sodium phosphate, pH 7.0, the spot was washed with 0.05% Triton X100 and 0.15 M NaCl (150 μl, total). Fig. 20C: In addition to 20 mM sodium phosphate, pH 7.0, the spot was washed with 100 mM imidazole, 0.15 M NaCl (150 μl total).

Figs. 21A-21D show the results of a comparison between components in normal human serum and diseased serum. Fig. 21A: Retentate map of normal serum on an adsorbent array Cu(II) site. Fig. 21B: Retentate map of disease serum on an adsorbent array Cu(II) site. Fig. 21C: Retained analytes of both serum samples are combined in an overlay fashion. To simplify the presentation, each peak of retained analyte is converted to a bar, the dashed bars represent analytes retained from a normal serum, and the solid bars represent analytes retained from a disease serum. Fig. 21D: To differentiate more clearly the difference between the two samples, a comparison plot is generated, where the ratio of the retained analytes from the samples are calculated and displayed. The two analytes marked with "*" show significant increases in the disease serum (5 to 10 fold increases).

Figs. 22A-22D show a comparison of retentate maps for control, diseased and drug-treated mouse urine on a Cu(II) adsorbent, and quantitation of amount of a marker in diseased and drug-treated urine.

Figs. 23A-23D show retentate maps of analytes in urine from four human cancer patients shown in "gel view" format. Difference maps between patients 1, 2 and 3 show two common analytes that are present in increased amounts in these patients.

Figs. 24A-24E show detection of M13 phage by laser desorption mass spectrometry through the detection of the gene VIII coat protein. The dilutions of the original 10^{12} phage per ml range from 1:10 to 1:100,000,000.

Figs. 25A-25B show the capture of M13 by desorption spectrometry using anti-M13 antibody as an adsorbent. Fig. 22A shows captured M13 phage with peaks representing gene VIII and gene III proteins. Fig. 22B is a control showing peaks representing the antibody adsorbent (singly and doubly charged).

Figs. 26A-26D show adsorption of M13 phage bearing an anti-tat single chain antibody by tat protein adsorbent. Single strength is shown under phage dilutions from 1:10 to 1:10,000.

Figs. 27A-27B show retention maps of TGF-β binding to docked TGF-β receptor fusion protein at 1 μg/ml (Fig. 27A) and at 100 ng/ml (Fig. 27B). The solid line shows binding without the presence of free TGF-β receptor. The dashed line shows binding in the presence TGF-β receptor.

Figs. 28 to 31 show the resolving power of retentate chromatography. Figs. 28A-28C show resolution of proteins from *Hemophilus* lysate using hydrophobic, cationic and Cu(II) adsorbents at molecular masses from 0 kD to 30 kD. Each retained analyte is represented by a bar, the height of the bar represents the intensity of the retained analyte. Figs. 29A-29C show resolution of proteins from *Hemophilus* lysate using hydrophobic, cationic and Cu(II) adsorbents at molecular masses from about 30 kD to about 100 kD. Fig. 30 shows combined resolution from 0 kD to 30 kD of *Hemophilus* proteins from each of the three adsorbents. Fig. 31 shows combined resolution from 20 kD to 100 kD of *Hemophilus* proteins from each of the three adsorbents.

Fig. 32 shows the binding of GST fusion protein to a normal adsorbent.

Figs. 33A-33B show binding of a specific ligand to GST fusion receptor docked to an adsorbent array (Fig. 33A) and lack of binding of the ligand to a control array that does not include the GST fusion receptor (Fig. 33B).

DETAILED DESCRIPTION OF THE INVENTION

I. DEFINITIONS

Unless defined otherwise, all technical and scientific terms used herein have the meaning commonly understood by a person skilled in the art to which this invention belongs. The following references provide one of skill with a general definition of many of the terms used in this invention: Singleton et al., DICTIONARY OF MICROBIOLOGY AND MOLECULAR BIOLOGY (2d ed. 1994); THE CAMBRIDGE DICTIONARY OF SCIENCE AND TECHNOLOGY (Walker ed., 1988); THE GLOSSARY OF GENETICS, 5TH ED., R. Rieger et al. (eds.), Springer Verlag (1991); and Hale & Marham, THE HARPER COLLINS DICTIONARY OF BIOLOGY (1991). As used herein, the following terms have the meanings ascribed to them unless specified otherwise.

"Analyte" refers to a component of a sample which is desirably retained and detected. The term can refer to a single component or a set of components in the sample.

"Adsorbent" refers to any material capable of adsorbing an analyte. The term "adsorbent" is used herein to refer both to a single material ("monoplex adsorbent") (e.g., a compound or functional group) to which the analyte is exposed, and to a plurality of different materials ("multiplex adsorbent") to which a sample is exposed. The adsorbent materials in a multiplex adsorbent are referred to as "adsorbent species." For example, an addressable location on a substrate can comprise a multiplex adsorbent characterized by many different adsorbent species (e.g., anion exchange materials, metal chelators, or antibodies), having different binding characteristics.

"Adsorb" refers to the detectable binding between an absorbent and an analyte either before or after washing with an eluant (selectivity threshold modifier).

"Substrate" refers to a solid phase to which an adsorbent is attached or deposited.

"Binding characteristic" refers to a chemical and physical feature that dictates the attraction of an adsorbent for an analyte. Two adsorbents have different binding characteristics if, under the same elution conditions, the adsorbents bind the same analyte with different degrees of affinity. Binding characteristics include, for example, degree of salt-promoted interaction, degree of hydrophobic interaction, degree of hydrophilic interaction, degree of electrostatic interaction, and others described herein.

"Binding conditions" refer to the binding characteristics to which an analyte is exposed.

"Eluant" refers to an agent, typically a solution, that is used to mediate adsorption of an analyte to an adsorbent. Eluants also are referred to as "selectivity threshold modifiers."

"Elution characteristic" refers to a feature that dictates the ability of a particular eluant (selectivity threshold modifier) to mediate adsorption between an analyte and an absorbent. Two eluants have different elution characteristics if, when put in contact with an analyte and adsorbent, the degree of affinity of the analyte for the adsorbent differs. Elution characteristics include, for example, pH, ionic strength,

modification of water structure, detergent strength, modification of hydrophobic interactions, and others described herein.

"Elution conditions" refer to the elution characteristics to which an analyte is exposed.

"Selectivity characteristic" refers to a feature of the combination of an adsorbent having particular binding characteristics and an eluant having particular elution characteristics that dictate the specificity with which the analyte is retained to the adsorbent after washing with the eluant.

"Selectivity conditions" refer to the selectivity characteristics to which an analyte is exposed.

"Basis for attraction" refers to the chemical and/or physico-chemical properties which cause one molecule to be attracted to another.

"Strength of attraction" refers to the intensity of the attraction of one molecule for another (also known as affinity).

"Resolve," "resolution," or "resolution of analyte" refers to the detection of at least one analyte in a sample. Resolution includes the detection of a plurality of analytes in a sample by separation and subsequent differential detection. Resolution does not require the complete separation of an analyte from all other analytes in a mixture. Rather, any separation that allows the distinction between at least two analytes suffices.

"High information resolution" refers to resolution of an analyte in a manner that permits not only detection of the analyte, but also at least one physico-chemical property of the analyte to be evaluated, e.g., molecular mass.

"Desorption spectrometry" refers to a method of detecting an analyte in which the analyte is exposed to energy which desorbs the analyte from a stationary phase into a gas phase, and the desorbed analyte or a distinguishable portion of it is directly detected by a detector, without an intermediate step of capturing the analyte on a second stationary phase.

"Detect" refers to identifying the presence, absence or amount of the object to be detected.

"Retention" refers to an adsorption of an analyte by an adsorbent after washing with an eluant.

"Retention data" refers to data indicating the detection (optionally including detecting mass) of an analyte retained under a particular selectivity condition.

"Retention map" refers to a value set specifying retention data for an analyte retained under a plurality of selectivity conditions.

"Recognition profile" refers to a value set specifying relative retention of an analyte under a plurality of selectivity conditions.

"Complex" refers to analytes formed by the union of 2 or more analytes.

"Fragment" refers to the products of the chemical, enzymatic, or physical breakdown of an analyte. Fragments may be in a neutral or ionic state.

"Differential expression" refers to a detectable difference in the qualitative or quantitative presence of an analyte.

"Biological sample" refers to a sample derived from a virus, cell, tissue, organ or organism including, without limitation, cell, tissue or organ lysates or homogenates, or body fluid samples, such as blood, urine or cerebrospinal fluid.

"Organic biomolecule" refers to an organic molecule of biological origin, e.g., steroids, amino acids, nucleotides, sugars, polypeptides, polynucleotides, complex carbohydrates or lipids.

"Small organic molecule" refers to organic molecules of a size comparable to those organic molecules generally used in pharmaceuticals. The term excludes organic biopolymers (e.g., proteins, nucleic acids, etc.). Preferred small organic molecules range in size up to about 5000 Da, up to about 2000 Da, or up to about 1000 Da.

"Biopolymer" refers to a polymer of biological origin, e.g., polypeptides, polynucleotides, polysaccharides or polyglycerides (e.g., di- or tri-glycerides).

"Polypeptide" refers to a polymer composed of amino acid residues, related naturally occurring structural variants, and synthetic non-naturally occurring analogs thereof linked via peptide bonds, related naturally occurring structural variants, and synthetic non-naturally occurring analogs thereof. Synthetic polypeptides can be synthesized, for example, using an automated polypeptide synthesizer. The term "protein" typically refers to large polypeptides. The term "peptide" typically refers to short polypeptides.

"Polynucleotide" refers to a polymer composed of nucleotide units. Polynucleotides include naturally occurring nucleic acids, such as deoxyribonucleic acid ("DNA") and ribonucleic acid ("RNA") as well as nucleic acid analogs. Nucleic acid analogs include those which include non-naturally occurring bases, nucleotides that engage in linkages with other nucleotides other than the naturally occurring

phosphodiester bond or which include bases attached through linkages other than phosphodiester bonds. Thus, nucleotide analogs include, for example and without limitation, phosphorothioates, phosphorodithioates, phosphorotriesters, phosphoramidates, boranophosphates, methylphosphonates, chiral-methyl phosphonates, 2-O-methyl ribonucleotides, peptide-nucleic acids (PNAs), and the like. Such polynucleotides can be synthesized, for example, using an automated DNA synthesizer. The term "nucleic acid" typically refers to large polynucleotides. The term "oligonucleotide" typically refers to short polynucleotides, generally no greater than about 50 nucleotides. It will be understood that when a nucleotide sequence is represented by a DNA sequence (i.e., A, T, G, C), this also includes an RNA sequence (i.e., A, U, G, C) in which "U" replaces "T."

"Detectable moiety" or a "label" refers to a composition detectable by spectroscopic, photochemical, biochemical, immunochemical, or chemical means. For example, useful labels include ^{32}P, ^{35}S, fluorescent dyes, electron-dense reagents, enzymes (e.g., as commonly used in an ELISA), biotin-streptavadin, dioxigenin, haptens and proteins for which antisera or monoclonal antibodies are available, or nucleic acid molecules with a sequence complementary to a target. The detectable moiety often generates a measurable signal, such as a radioactive, chromogenic, or fluorescent signal, that can be used to quantitate the amount of bound detectable moiety in a sample. The detectable moiety can be incorporated in or attached to a primer or probe either covalently, or through ionic, van der Waals or hydrogen bonds, e.g., incorporation of radioactive nucleotides, or biotinylated nucleotides that are recognized by streptavadin. The detectable moiety may be directly or indirectly detectable. Indirect detection can involve the binding of a second directly or indirectly detectable moiety to the detectable moiety. For example, the detectable moiety can be the ligand of a binding partner, such as biotin, which is a binding partner for streptavadin, or a nucleotide sequence, which is the binding partner for a complementary sequence, to which it can specifically hybridize. The binding partner may itself be directly detectable, for example, an antibody may be itself labeled with a fluorescent molecule. The binding partner also may be indirectly detectable, for example, a nucleic acid having a complementary nucleotide sequence can be a part of a branched DNA molecule that is in turn detectable through hybridization with other labeled nucleic acid molecules. (See, e.g., PD. Fahrlander and A. Klausner,

Bio/Technology (1988) 6:1165.) Quantitation of the signal is achieved by, e.g., scintillation counting, densitometry, or flow cytometry.

"Plurality" means at least two.

"Purify" or "purification" means removing at least one contaminant from the composition to be purified. Purification does not require that the purified compound be 100% pure.

A "ligand" is a compound that specifically binds to a target molecule.

A "receptor" is compound that specifically binds to a ligand.

"Antibody" refers to a polypeptide ligand substantially encoded by an immunoglobulin gene or immunoglobulin genes, or fragments thereof, which specifically binds and recognizes an epitope (e.g., an antigen). The recognized immunoglobulin genes include the kappa and lambda light chain constant region genes, the alpha, gamma, delta, epsilon and mu heavy chain constant region genes, and the myriad immunoglobulin variable region genes. Antibodies exist, e.g., as intact immunoglobulins or as a number of well characterized fragments produced by digestion with various peptidases. This includes, e.g., Fab' and $F(ab)'_2$ fragments. The term "antibody," as used herein, also includes antibody fragments either produced by the modification of whole antibodies or those synthesized *de novo* using recombinant DNA methodologies. It also includes polyclonal antibodies, monoclonal antibodies, chimeric antibodies and humanized antibodies. "Fc" portion of an antibody refers to that portion of an immunoglobulin heavy chain that comprises one or more heavy chain constant region domains, CH_1, CH_2 and CH_3, but does not include the heavy chain variable region.

A ligand or a receptor (e.g., an antibody) "specifically binds to" or "is specifically immunoreactive with" a compound analyte when the ligand or receptor functions in a binding reaction which is determinative of the presence of the analyte in a sample of heterogeneous compounds. Thus, under designated assay (e.g., immunoassay) conditions, the ligand or receptor binds preferentially to a particular analyte and does not bind in a significant amount to other compounds present in the sample. For example, a polynucleotide specifically binds under hybridization conditions to an analyte polynucleotide comprising a complementary sequence; an antibody specifically binds under immunoassay conditions to an antigen analyte bearing an epitope against which the antibody was raised; and an adsorbent specifically binds to an analyte under proper elution conditions.